Cry1Eb, Cry1Fa, Cry1Fb, Cry1Hb, Cry1Ia, Cry1Ib, Cry1Ja, and Cry1Jb crystal proteins being highly preferred.

These loop region mutations may include changing any one or more amino acids to any other amino acid, so long as the resulting protein has increased Lepidopteran insecticidal activity. The inventors have shown that exemplary substitutions such as changing one or more arginine residues to any other amino acid results in polypeptides having increased insecticidal activity. Particularly preferred substitutions of arginine residues include those substituted by alanine, leucine, methionine, glycine or aspartic acid. Likewise, the inventors have shown that substitution of lysine residues by any other amino acid, such as an alanine residue, also results in insecticidally-activetoxins. Indeed any such modification is contemplated by the inventors to be useful, so long as the substitution, addition, deletion, or modification of one or more of the amino acid residues in the preferred loop region results in a polypeptide which has improved insecticidal activity when compared to an unmodified Cry1 polypeptide. The inventors contemplate that combinatorial mutants as described herein will find particular use in the generation of a polypeptide having one or more mutations in multiple loop regions, or alternatively, in the generation of a polypeptide having multiple mutations with a single loop region. Such combinatorial mutants, as the inventors have shown herein often result in mutagenized polypeptides which have significantly improved insecticidal activity over the wild-type unmodified sequence.

Of course, one of skill in the art will realize that these amino acid modifications need not be made in the polypeptides themselves (although chemical synthesis of such polypeptides is well-known to those of skill in the art), but may also be made via mutagenesis of a nucleic acid segment which encodes such a polypeptide. Means for such DNA mutagenesis are described herein in detail, and exemplary polypeptides constructed using such methods are described in detail in the Examples which follow herein.

2.1 MUTAGENIZED CRY1 GENES AND POLYPEPTIDES

Accordingly, the present invention provides mutagenized Cry1C protein genes and methods of making and using such genes. As used herein the term "mutagenized

Cry1C protein gene(s)" means one or more genes that have been mutagenized or altered to contain one or more nucleotide sequences which are not present in the wild type sequences, and which encode mutant Cry1C crystal proteins (Cry1C*) showing improved insecticidal activity. Preferably the novel sequences comprise nucleic acid sequences in which at least one, and preferably, more than one, and most preferably, a significant number, of wild-type Cry1C nucleotides have been replaced with one or more nucleotides, or where one or more nucleotides have been added to or deleted from the native nucleotide sequence for the purpose of altering, adding, or deleting the corresponding amino acids encoded by the nucleic acid sequence so mutagenized. The desired result, therefore, is alteration of the amino acid sequence of the encoded crystal protein to provide toxins having improved or altered activity and/or specificity compared to that of the unmodified crystal protein. Modified *cry1C* gene sequences have been termed *cry1C** by the inventors, while modified Cry1C crystal proteins encoded therein are termed Cry1C* proteins.

Contrary to the teachings of the prior art which have focused attention on the α-helices of crystal proteins as sites for genetic engineering to improve toxin activity, the present invention differs markedly by providing methods for creating modified loop regions *between* adjacent α-helices within one or more of the protein's domains. In a particular illustrative embodiment, the inventors have shown remarkable success in generating toxins with improved insecticidal activity using these methods. In particular, the inventors have identified unique loop regions within domain 1 of a Cry1 crystal protein which have been targeted for specific and random mutagenesis.

In a preferred embodiment, the inventors have identified the predicted loop regions between α-helices 1 and 2a; α-helices 2b and 3; α-helices 3 and 4; α-helices 4 and 5; α-helices 5 and 6, α-helices 6 and 7; and between α-helix 7 and β-strand 1 in Cry1 crystal proteins. Using Cry1C as an exemplary model, the inventors have generated amino acid substitutions within or adjacent to these predicted loop regions to produce synthetically-modified Cry1C* toxins which demonstrated improved insecticidal activity. In mutating specific residues within these loop regions, the inventors were able to

produce synthetic crystal proteins which retained or possessed enhanced insecticidal activity against certain lepidopteran pests, including the beet armyworm, S. *exigua*.

Claimed is an isolated *B. thuringiensis* crystal protein that has one or more modified amino acid sequences in one or more loop regions of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2. These synthetically-modified crystal proteins have insecticidal activity against Lepidopteran insects. The modified amino acid sequences may occur in one or more of the following loop regions: between α helices 1 and 2a, α helices 2b and 3, α helices 3 and 4, α helices 4 and 5, α helices 5 and 6, α helices 6 and 7 of domain 1, or between the α helix 7 of domain 1 and β strand 1 of domain 2.

In an illustrative embodiment, the invention encompasses modifications which may be made in or immediately adjacent to the loop region between α helices 1 and 2a of a Cry1C protein. This loop region extends from about amino acid 42 to about amino acid 46, with adjacent amino acids extending from about amino acid 39 to about amino acid 41 and from about amino acid 47 to about amino acid 49.

The invention also encompasses modifications which may be made in or immediately adjacent to the loop region between α helices 2b and 3 of a Cry1C protein. This loop region extends from about amino acid 84 to about amino acid 88, with adjacent amino acids extending from about amino acid 81 to about amino acid 83, and from about amino acid 89 to about amino acid 91.

The invention also encompasses modifications which may be made in or immediately adjacent to the loop region between α helices 3 and 4 of a Cry1C protein. This loop region extends from about amino acid 119 to about amino acid 123, with the adjacent amino acids extending from about amino acid 116 to about amino acid 118, and from about amino acid 124 to about amino acid 126.

Likewise, the invention also encompasses modifications which may be made in or immediately adjacent to the loop region between α helices 4 and 5 of a Cry1C protein. This loop region extends from about amino acid 149 to about amino acid 155, with the adjacent amino acids extending from about amino acid 146 to about amino acid 148, and from about amino acid 156 to about amino acid 158.